PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference JWJ01009WO	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2004/001673	International filing date *(day/month/year)* 16.04.2004	Priority date *(day/month/year)* 16.04.2003
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant LINGVITAE AS et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand 11.11.2004	Date of completion of this report 18.08.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Reuter, U Telephone No. +31 70 340-1036

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/001673

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-23 as originally filed

Claims, Numbers

1-15 as originally filed

Drawings, Sheets

1-6 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6,9,10,12-15
	No:	Claims	1-5,7,8,11
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-15
Industrial applicability (IA)	Yes:	Claims	1-15
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

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Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item V.
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 The following documents are referred to in this communication:

D1 : WO 01/61036 A (TOWLER PHILIP DEAN ;LEXOW PREBEN (NO); COMPLETE GENOMICS AS (NO)) 23 August 2001 (2001-08-23)
D2 : WO 00/39333 A (JONES ELIZABETH LOUISE ;LEXOW PREBEN (NO)) 6 July 2000 (2000-07-06)
D3 : US 5 714 330 A (BRENNER SYDNEY ET AL) 3 February 1998 (1998-02-03)
D4 : EP 0 701 001 A (HITACHI LTD) 13 March 1996 (1996-03-13)
D5 : US 6 258 533 B1 (JONES DOUGLAS H) 10 July 2001 (2001-07-10)

2 **Novelty** (Article 33(2) PCT)

2.1 D1 discloses a method for sequencing in which nucleic acid target molecules are attached to a solid support, treated with an endonuclease, and the sequence of the created overhanging ends is detected with the help of labelled adapters ("signal sequence") that are detected after treatment with a ligase ("molecule that interacts") (p. 32). Additionally a method of ligating two adapters, one being biotinylated ("signal sequence"), to both ends of the target sequence is disclosed (example 3). The unligated target sequences do not bind to strepavidin and are separated. A primer binding to an adapter sequence is used to amplify the ligation products. The amplified ligation products are detected on an array. D1 thus discloses a method for identifying specific characteristics of a target polynucleotide that comprises the steps of (I) attaching to one end of each target nucleotide a signal sequence (hybridisation of labelled adaptors, p. 32, example 3), (ii) contacting the target polynucleotides with a

molecule that interacts if the target polynucleotide if the characteristic is present (ligase ligates adaptor to target if target has an appropriate overhang), (iii) separating those target polynucleotides that interact (e.g. via biotin in example 3), and (iv) identifying which signal sequences are present (label detection (p. 32) or PCR (example 3)). In the light of D1 claims 1-5,7,8, and 11 do not meet the requirements of the PCT in respect of novelty (Article 33(2)).

2.2 D2 discloses the use of immobilized adapters to sort and sequence nucleic acids (p. 33-34). Having bound to the adapter (attachment of the signal sequence) the target molecules are contacted with a ligase (molecule that interacts if the adapter is bound and thus the characteristic is present). After separation of the unligated molecules the target molecules ligated to the adaptors are detected. A variation of the method with not immobilized adapters is described on page 21 and in example 1 of D2. The steps of the method can be repeated. In the light of D2 claims 1 and 3 do not meet the requirements of the PCT in respect of novelty (Article 33(2)).

2.3 D3 discloses a sequencing method in which target nucleic acids are treated with endonucleases and ligated to adapters that are immobilized on beads (example 6, col. 17). The other end of the target is ligated to adaptors (signal sequence and interacting molecule) carrying a base specific label that is used to identify a nucleotide at one position of the target. The steps can be repeated by cleaving the adapter via a type II endonuclease restriction site (example 6). D3 additionally discloses the use of methylcytosine triphosphates to block nuclease recognition sites (col. 19). In the light of D3 claims 1,3 and 7 do not meet the requirements of the PCT in respect of novelty (Article 33(2)).

2.4 D4 discloses a DNA analysis method in which nucleic acid target molecules are labelled by ligation to a labelled oligonucleotide (signal sequence). The labelled products are bound to probes (interacting molecule) that are extended along the target molecules (col. 9-10, fig. 1). The molecules bound to non extended probes are separated and the target molecules attached to the extended probes are detected via the signal sequence. In the light of D4 claims 1,3 and 7 do not meet the requirements of the PCT in respect of novelty (Article 33(2)).

2.5 In the light of D1-D4 claims 1-5,7,8, and 11 do not meet the requirements of the PCT in respect of novelty (Article 33(2)).

3 **Inventive Step** (Article 33(3) PCT)

3.1 The document D2 is regarded as being the closest prior art to the subject-matter of claim 13, and discloses the use of immobilized adapters to sequence nucleic acids (p. 33-34). The method of D2 comprises the steps of (I) treating a sample to create overhangs which are to be sequenced that have a defined number of bases, (ii) dividing the sample and contacting each separate sample with (immobilized) adapters that comprise overhangs that are complementary to the overhang being sequenced or sorted (p. 33), and detecting the binding of the fragment to the adapter e.g. with a dye (p. 83). Having bound to the adapter, the target molecules are contacted with a ligase. The steps of the method can be repeated with the help of target sites for type II endonucleases in the adaptor sequence.

3.2 The subject-matter of claim 13 therefore differs from this known from D2 in that the binding of the target to the sequence specific adapter is detected with the help of a second adaptor that is ligated to the second end of the target molecule and a subsequent amplification step using primers that hybridise to the adapter sequences. Claim 13 does not comprise the feature that the attachment of the signal sequence is performed before the target fragment is contacted with the adapter that binds to the overhang that is to be sequenced.

3.3 The problem to be solved by the present invention may therefore be regarded as providing an alternative method for the detection of the binding of a base specific adapter to its target sequence.

3.4 The solution proposed in claim 13 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

3.5 D5 discloses the use of a polymerase reaction to detect a base specific adapter

ligation with primers that correspond to the ligated adapters (col. 25) and discloses the use of two adapters and corresponding primers to analyse target nucleic acids (col. 32). The use of primers that hybridise to adapter sequences is also disclosed in D2 (p. 46, first paragraph). Being confronted with the problem to be solved the person skilled in the art would, in the light of D2 and D5, modify the method of D2 to arrive at the claimed invention without an inventive step. Consequently the subject-matter of claim 13 does not involve an inventive step in the sense of Article 33(3) PCT.

3.6 Dependent claims 6,9,10,12,14, and 15 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step. (see documents D1-D5 and the corresponding passages cited in the search report).

3.7 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-15 does not involve an inventive step in the sense of Article 33(3) PCT.